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MATURATION OF THE REPRODUCTIVE TRACT OF *ARION ATER* (PULMONATA: ARIONIDAE)

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ABSTRACT

The maturation of the reproductive tract of the garden slug *Arion ater* Linnaeus was studied in detail and the possible factors influencing this maturation were investigated.

Slugs were collected from a natural population at regular intervals throughout the breeding season and divided into weight groups; the reproductive tracts were dissected out and the various parts weighed and classified histologically into maturation stages.

Field results showed that very few animals manage to over-winter after breeding; but that they rather do so in the egg or very young stages. Growth takes place throughout the year and there is always a wide range of body sizes in any given population. In Britain copulation occurs from July to September and is followed shortly by laying. A large decrease is then seen in field numbers, probably due to post-laying mortality.

The various parts of the reproductive tract can readily be divided into a number of easily recognisable maturation stages: Hermaphrodite gland: 9 stages; albumen gland: 4 stages; common duct: 7 stages; genital atria, epiphallus and spermatheca: 6 stages, which are described in detail. It was shown that a general relationship exists between the maturation of the tract and the season and that the maturation of the hermaphrodite gland is correlated to the maturation of the remainder of the tract. By the "Student's t" test, the ratios of the reproductive tract region weight/body weight for each maturation stage was found to be significantly different, showing that these groupings are real. Finally, contrary to the suggestion of Lusis (1961), there was little relationship between maturation and body size.

Animals were also collected from the same natural population, subjected to different controlled environmental factors and compared to animals from the natural population. They were subjected to nearly constant temperatures of 4°C, 10°C, 18°C and 25°C for varying periods of time and also to artificial light for 8 hrs./day, 16 hrs./day and continuously; to natural day-length light and no light. Although the reproductive cycle could be advanced or retarded by these environmental factors the relationship between the maturation stage of the hermaphrodite gland and the remainder of the tract could not be altered. Animals hatched and reared in the laboratory showed a gross variation in their maturation, possibly due to some major upset in their control mechanism.

Three phases in maturation of the reproductive tract of *Arion ater* can be distinguished:

1. Differentiation of the male glands, initiated by the general rise in temper-

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ature in spring.

2. Copulation and differentiation of the female glands, which occurs during the mid-spermatozoa stage of the hermaphrodite gland between July and September and is the "critical point" in maturation. A "trigger" mechanism of control, probably of a neurosecretory nature, is postulated, as already suggested (Smith, 1964).

3. Fertilization, laying and onset of atrophy, possibly at a standard period after copulation and depending on general metabolic rate.

INTRODUCTION

In all animals that have a breeding season there must be some means by which all the members of a given population come into breeding condition at the same time. Such a coordination necessitates the existence of at least one control mechanism, probably of many links, related to a seasonal environmental change. In a complex hermaphrodite animal such as the garden slug *Arion ater* Linnaeus it also means that the control mechanism must be able to either synchronize the maturation of all the different reproductive glands directly or trigger off a synchronized pattern of maturation.

The structure of the mature reproductive tract and the histochemistry of its glandular secretions in *Arion ater* have been discribed by Lusis (1961) and Smith (1964, 1965). Lusis's suggestion that the reproductive activity was mainly related to age and body size conflicted with preliminary observations made at the University College of North Wales. Consequently the present work was undertaken to describe in detail the stages in the maturation of the reproductive tract of *Arion ater* and to investigate the possible factors influencing this maturation.

MATERIALS AND METHODS

Animals were collected from the grounds of the University College of North Wales, Bangor, North Wales, once a fortnight from March to November, 1963. Bait traps of cabbage leaves were visited with a flashlight 2-3 hours after sunset and all the *Arion ater* from each trap were collected and left in a glass covered plastic bowl in the laboratory overnight. Next day they were divided into 2 gm weight groups and counted. From each collection 10-12 slugs were taken for dissection, the ratio of the weight groups in the sample being the same as that in the total collection. These slugs were then weighed individually, killed by injection with fixative (Susa or Elftman-Dichromate-Sublimate), and the whole reproductive tract was rapidly dissected out and fixed in either Susa for 6 hours or in Elftman-Dichromate-Sublimate for 3 days (Elftman, 1957). They were then transferred to cellosolve where they were stored.

The fixed and stored hermaphrodite gland, albumen gland, common duct and genital atria were dried with filter paper, and weighed separately on a torsion balance. Half to 3/4 of these tracts, taking in the full range of body weights, were embedded in Ester Wax, sectioned at 10 μ and stained in Hemalum and Eosin.

The remainder of the animals from each collection were used for experiments to determine the effects of certain different environmental factors. This was done so that a direct comparison could be made between the experimental results and the natural maturation cycle. For one experiment animals were obtained from eggs which were laid and hatched in the laboratory. For all the experiments animals were kept in glass covered plastic bowls with damp sphagnum; the bowls were cleaned out and the sphagnum changed regularly. The slugs were fed on fresh lettuce, carrot and moistened crushed "rat cubes"

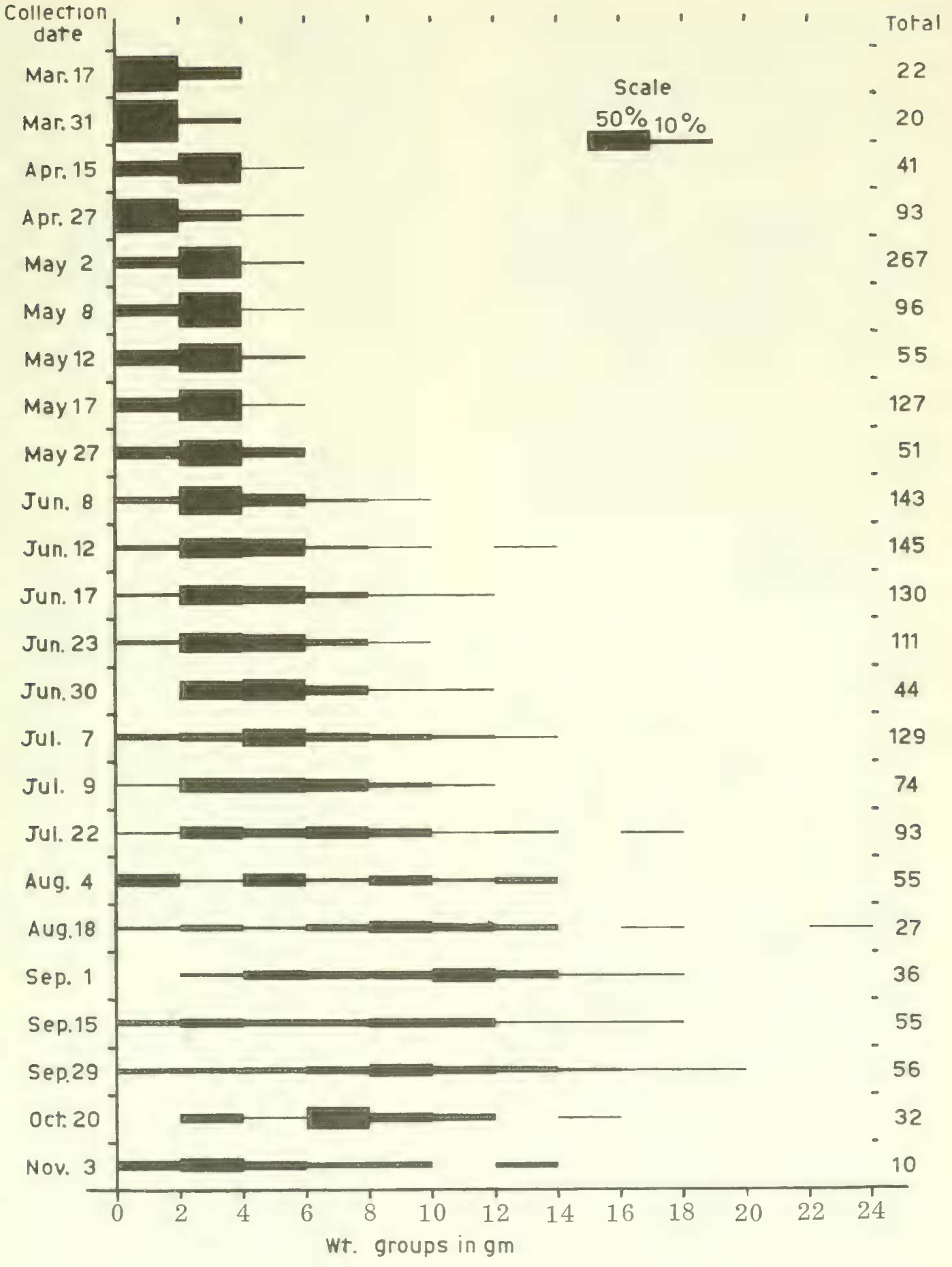

FIG. 1. Histogram showing the percentages of animals in each 2 gm weight group and the total number of animals in each collection.

each day. Moribund animals were removed from the bowls as soon as they were noticed, but this did not prevent some cannibalism. Animals were killed, fixed, dissected and weighed as described above at the end of the experiments. The following experimental conditions were applied:

1) Temperature. Temperatures were maintained approximately constant within ±2°C of the level required. The 4°C and 10°C experiments were carried out in 2 cold constant temperature rooms; the 18-20°C experiments were carried out in the heated laboratory or animal house; and the 25°C temperature was maintained in a large cabinet heated by shaded light bulbs, the temperature being regulated by a thermostat and a large fan.

2) Illumination. The usual day lengths for experiments were either the natural day or a constant 16 hour day. Artificial illumination was mainly by 60 watt daylight blue bulbs approximately 45 cm above the bowls; the day length being regulated by an automatic time switch. For complete darkness the outside of the bowl and glass was painted black and covered with a large black cloth.

FIELD COLLECTION RESULTS

The percentage of slugs in each 2 gm weight group in each collection is shown in Fig. 1.

The first collections taken in March 1963 were all young animals--probably not long hatched. The complete absence of large animals from the previous year may be atypical and due to the exceptionally severe winter; possibly the eggs or very young animals are far better able to stand extremes of weather conditions. No inference should be drawn from the widely differing total numbers collected at different collecting times, as on some occasions extra slugs were collected for use in experiments and these were included in these calculations for greater accuracy.

It is seen from Fig. 1 that, while growth takes place throughout the sea-

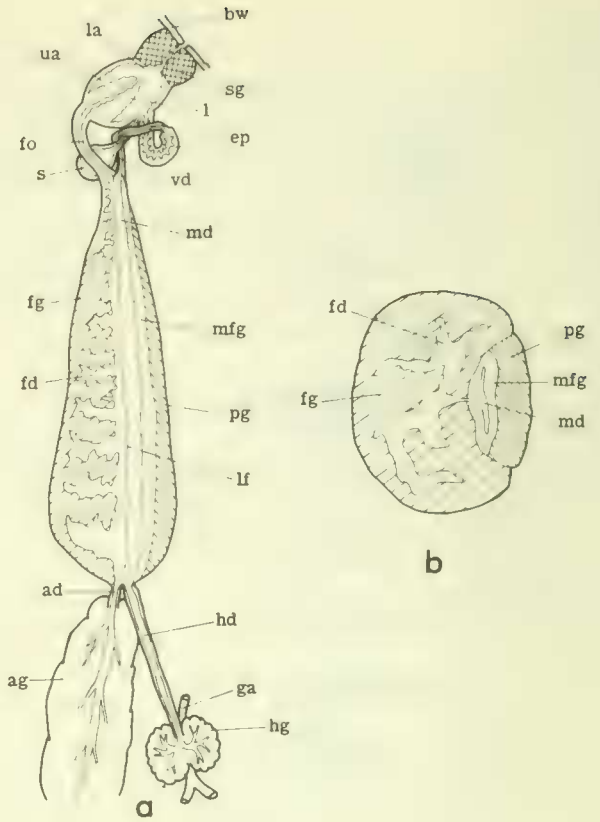

FIG. 2. a. Diagram of the reproductive tract of *Arion ater* showing the general structure and location of the glands. b. A transverse section through the proximal half of the common duct showing the distribution of the glands.

KEY TO ABBREVIATIONS

ad - albumen duct
ag - albumen gland
bw - body wall
cd - common duct
ep - epiphallus
fd - female duct
fg - female gland
fo - free oviduct
ga - genital artery
hd - hermaphrodite duct
hg - hermaphrodite gland
il - intermediate layer
l - ligula
la - lower atrium
lf - longitudinal fold
md - male duct
mfg - male flask gland
pg - prostate gland
s - spermatheca
sd - spermathecal duct
sg - spongy gland
ua - upper atrium
vd - vas deferens

son, there is always a very wide range of body sizes. Thus even in July and August there were still many very small slugs, although no mature slugs capable of laying eggs had been found earlier in the season. This suggests either a very late hatching of eggs laid the previous winter, or, as later results tend to confirm, a large variation in growth rate between individuals hatched at approximately the same time. The apparently significant large number of very small animals in early August therefore probably does not represent a small secondary breeding period.

The drop in the mean weights seen at the end of September and October is probably due to the onset of laying, when slugs lose a considerable amount of their body weight. The large percentage of very small animals seen on November 3rd is probably artificial since the total is only 10 animals. A few animals however were found that were exceedingly small and undeveloped and could only have been recently hatched, undoubtedly from eggs laid that season. At that time there was a very great decrease in the numbers of slugs to be found in the field, which may have been due to any number of reasons: the onset of cold weather, some possible change in behavior, or most likely a high incidence of post-laying mortality. I have observed the latter in animals kept in the laboratory many times, and it may be a natural phenomenon.

STAGES IN MATURATION OF THE REPRODUCTIVE TRACT

The general structure of the mature reproductive tract is shown in Fig. 2. Each part of the reproductive tract has a maturation cycle which is quite readily divided into a number of stages. These stages are described below, with special reference to the salient diagnostic features of each stage.

Hermaphrodite Gland Stages

A; Spermatogonia and Spermatocyte Stage

The gland consists of very small alveoli containing 3 types of cells. On the epithelium is a single layer of spermatogonia that are readily distinguished by their small size. In the lumina of the alveoli are many spermatocytes (larger cells) with large nuclei and a number of oocytes. There are also many spaces between these cells.

B; Early Spermatid Stage (Fig. 3a)

Amongst the spermatocytes which now fill the alveoli are seen small clumps of dividing cells, which give rise to spermatids by meiosis. Large numbers of dividing cells showing the various chromosome figures are characteristic for this stage. Nurse cells also differentiate and the oocytes are larger. More ducts and blood vessels seem to be present between the alveoli.

C; Late Spermatid Stage

The spermatid is now the predominant constituent, these cells being arranged in clumps around large unstained nurse cells or on the walls of the alveoli. A few spermatids show small tails. The oocytes vary in size, but a few are very large.

D; Early Spermatozoa Stage

Spermatids still predominate but there are a few clumps of fully formed sperm attached to darkly staining nurse cells. At this stage the gland has become very large with thin walls.

E; Mid-Spermatozoa Stage (Fig. 3b)

Sperm, mainly attached in clumps to nurse cells, predominate though a few small groups of spermatids remain. It is at this stage that sperm commence to be liberated into the hermaphrodite duct. Small amounts of granular black pigment

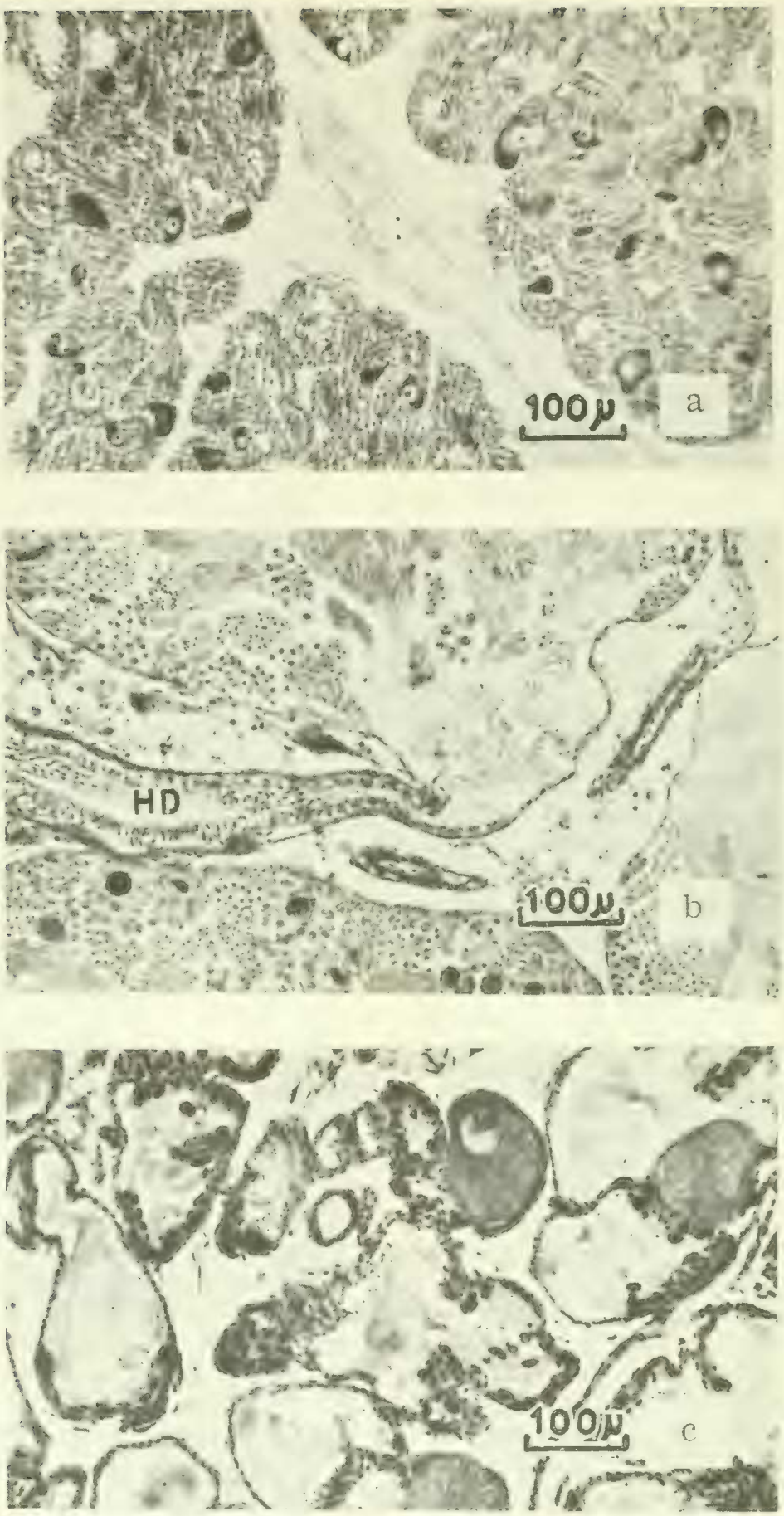

FIG. 3. Hermaphrodite gland of *Arion ater*. a. Early spermatid stage (B) showing spermatocytes and early oocytes. b. Mid-spermatozoa stage (E) showing the clumps of mature sperms in the alveolus. c. Late oocyte stage (H) showing the empty alveoli.

occur in the connective tissue.

F; Late Spermatozoa Stage

The lumina of the alveoli are full of mature, free sperm with no spermatogenesis stages present. The gland is smaller as some sperm have passed out to be stored in the distal part of the hermaphrodite duct. A few cells of the alveolar wall have started to enlarge. Oocytes are large and lightly stained.

G; Early Oocyte Stage

Only a few clumps of sperm now remain. Large lightly stained oocytes are now free in the lumina of the alveoli. The gland is smaller with a lot of black pigment in the walls of the alveoli. There is a fairly large amount of inter-alveolar tissue.

H; Late Oocyte Stage (Fig. 3c)

The lumina of the alveoli are almost filled by long cytoplasmic processes of enlarging epithelial cells. There are only a few free sperm and oocytes in the lumina. There is a lot of loose tissue, blood vessels and black pigment granules in the inter-alveolar spaces.

I; Atrophy Stage

The gland is very small and black, with no gametes present. The lumina of the alveoli are filled with enlarged epithelial cells. Large numbers of sudanophilic droplets were found associated with these processes and cells in fresh frozen sections.

Albumen Gland Stages

A; Simple Tubular Stage

The gland is extremely small and consists of a few loosely coiled tubules. These consist of a layer of cells arranged radially around a central duct.

B; Compound Tubular Stage (Fig. 4a)

The gland has become a little larger due to a great increase in the number of tubules. The cells too are larger but no secretion is present.

C; Secreting Stage (Fig. 4b)

The cells are now producing eosinophilic secretion which is being stored in the cytoplasm. The cell boundaries are however still distinct.

D; Mature Stage (Fig. 4c)

The cells are full of secretion with only the nuclei visible, the cell walls having been obliterated. The secretion in the fixed state appears as large polygonal granules.

Common Duct Stages

A; Undifferentiated Stage

The male and female ducts, separated by the longitudinal fold, are present and are both lined by tightly packed epithelial cells. The only other identifiable part is the prostate gland, which consists of a few coiled tubules of small radially arranged cells.

B; Early Male Stage (Fig. 5a)

The prostate gland has greatly enlarged by an increase in the number of tubules and the size of the cells. The male flask glands have differentiated and the cells of the male duct have enlarged.

C; Mid-Male Stage

All the male glands have completely differentiated and secretion is beginning to be produced and stored in them. The female duct is still undifferentiated.

D; Mature Male Stage (Fig. 5b)

The prostate gland cells are so full of eosinophilic secretory granules that the cell boundaries and lumina are indistinct. The male flask cells are also full of secretion. The walls of the female duct are slightly folded and a few female gland cells are starting to differentiate.

E; Early Female Stage (Fig. 5c)

Copulation has just occurred and the male glands have emptied their secretion into the male duct. Small vacuoles are seen in the reduced cells. The epi-

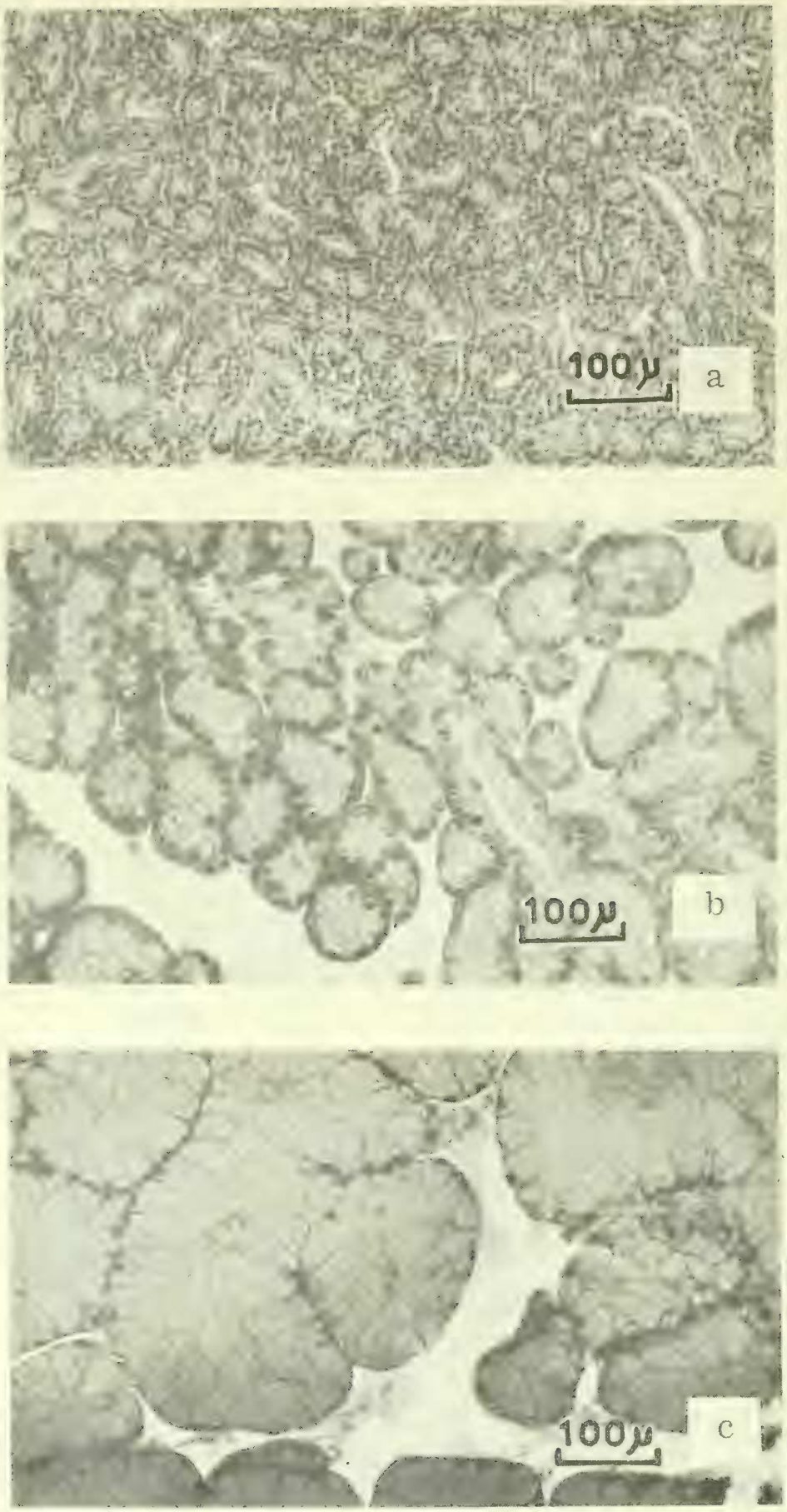

FIG. 4. Albumen gland of *Arion ater*. a. Compound tubule stage (B). b. Secreting stage (C) showing the cells greatly enlarged with secretion. c. Mature stage (D) showing the cells almost obliterated due to the stored secretion.

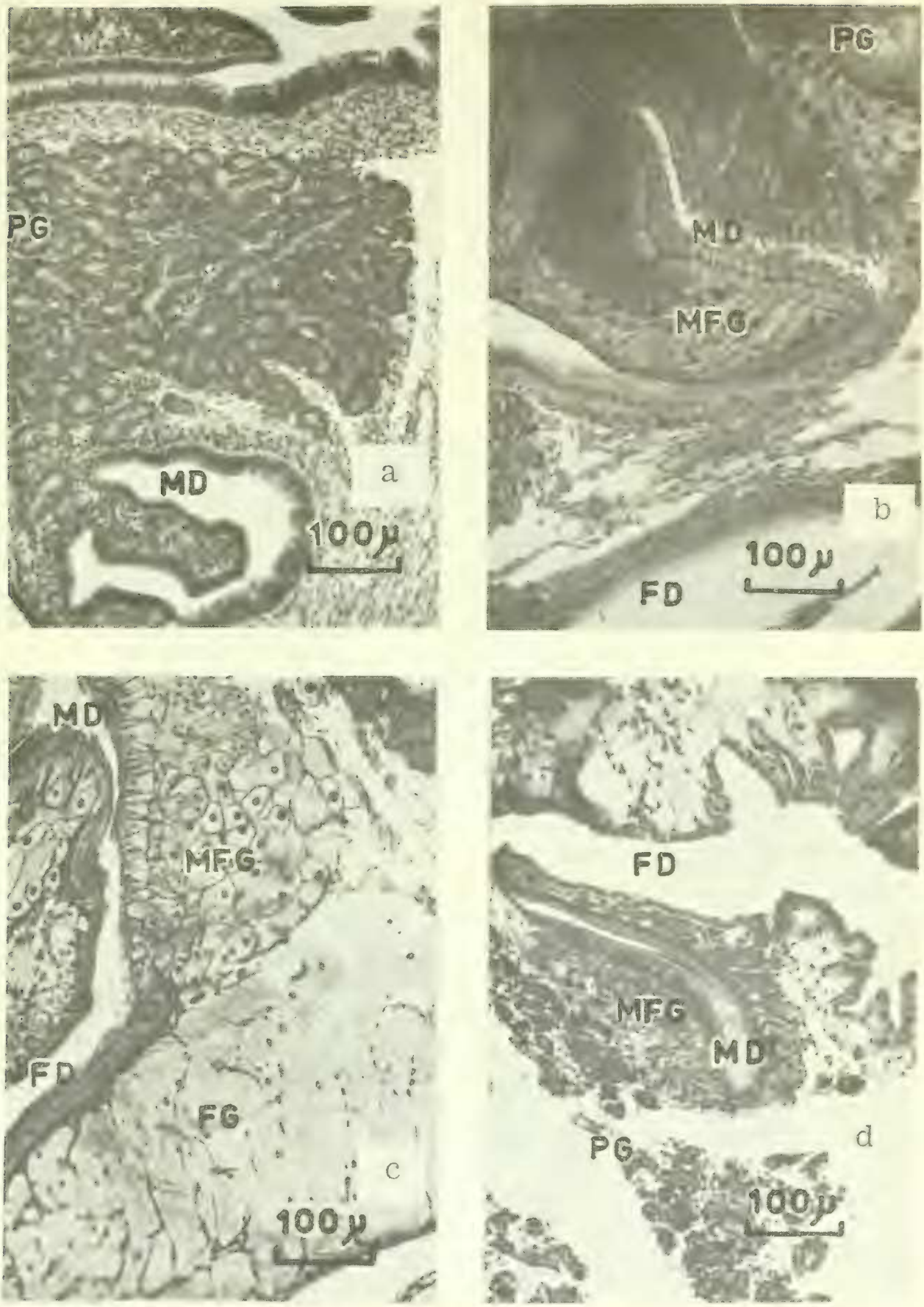

FIG. 5. Common duct of *Arion ater*. a. Early male stage (B). b. Mature male stage (D) showing the mature male and the undifferentiated female glands. c. Early female stage (E) showing the vacuolated male glands and the enlarging female glands. d. Atrophy stage (G) showing great reduction in the male and female glands.

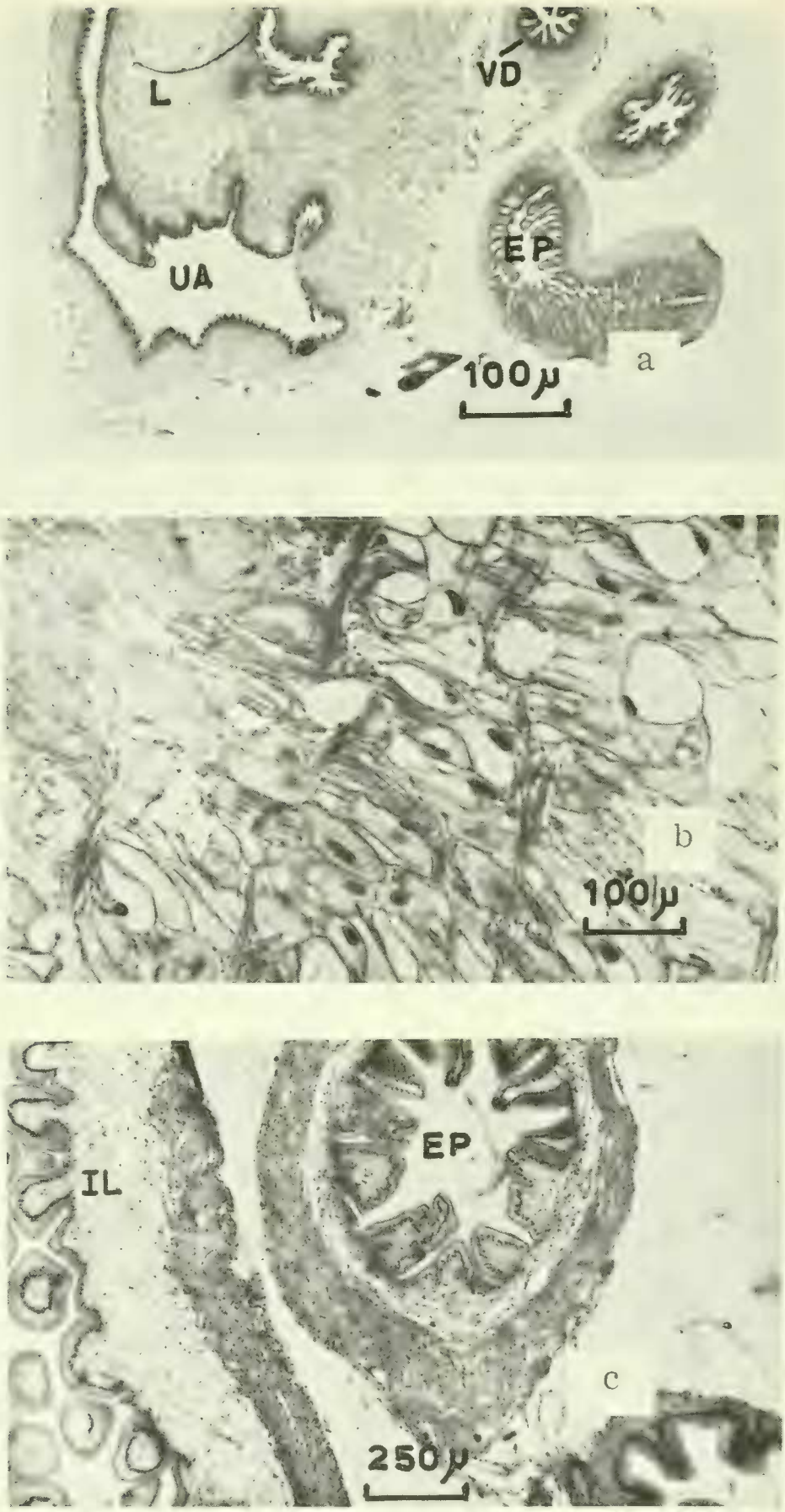

FIG. 6. a. Folding stage (B) of the genital atria and the early stage of the epiphallus, vas deferens and spermathecal duct. b. Vacuolated cells of the mature spongy gland. c. Regular folding of the epithelium of the epiphallus and also the intermediate layer of the mature epiphallus.

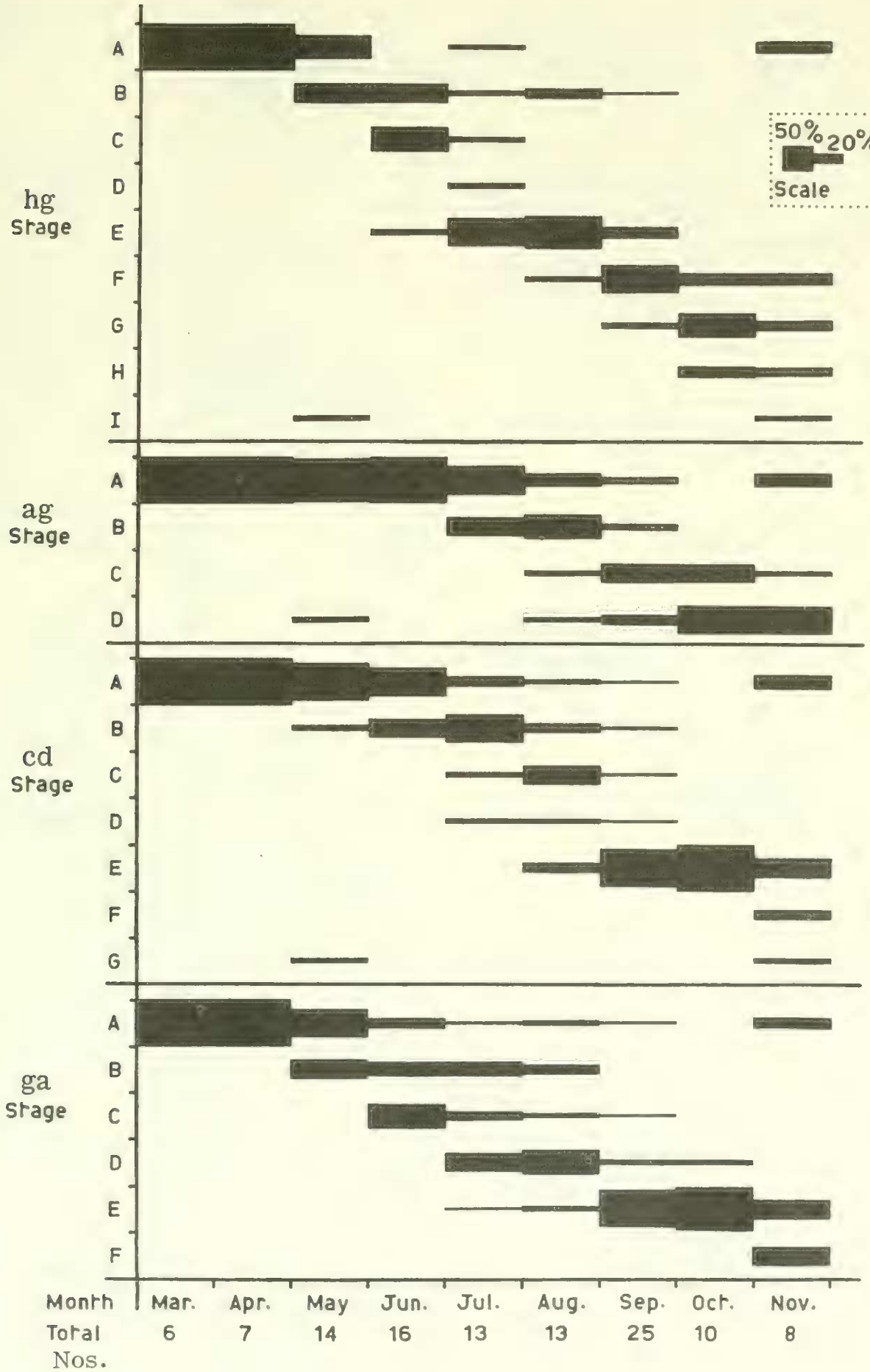

FIG. 7. Histogram showing the percentage of animals with each maturation stage of the hermaphrodite gland (hg), albumen gland (ag), common duct (cd) and genital atria (ga) for each month.

thelium of the female duct is cubical and the female gland cells have differentiated and are starting to enlarge with secretion.

F; Mature Female Stage

The female gland is greatly enlarged and full of secretion. Some have small vacuoles, the male glands are much reduced with small vacuolated cells.

G; Atrophy Stage (Fig. 5d)

All the gland cells of the tract are vacuolated and are much reduced in size. The male glands are more advanced in this degeneration and have become almost unrecognizable.

Stages in Genital Atria, Epiphallus and Spermatheca

A; Undifferentiated Stage

The atrium is a simple sac with a large clump of undifferentiated cells, the ligula, at one end. The Epiphallus is very small but shows its typical folding.

B; Folding Stage (Fig. 6a)

The walls of the organs differentiate and fibres begin to form. The epithelium of the atrium commences folding.

C; Enlarging Stage

The atrium is now enlarged and differentiated into the upper and lower atria. The cells of the spongy gland have started to differentiate. Both the epiphallus and spermatheca are also enlarging.

D; Mature Stage (Fig. 6b, c)

The atria are now fully formed and the spongy gland is enlarged and full of secretion (Fig. 6b). The intermediate layer of the epiphallus is differentiated and full of secretion (Fig. 6c). The spermatheca is large but empty.

E; Copulation Stage

The spermatheca is full of the partners' transferred spermatophore material and sperm after copulation. The epithelia of the atria, the intermediate layer of the epiphallus and the spongy gland have all secreted and are slightly vacuolated.

F; Atrophy Stage

The spongy gland is still very large. The other tissues and organs are smaller.

THE RELATIONSHIP OF MATURATION TO THE SEASON

The number of animals exhibiting each stage in the maturation of the genital tract, expressed as a percentage of the total animals for each month is illustrated in Fig. 7.

March and April: even though the animals are growing in size, the reproductive organs are in a completely undifferentiated state.

May: the animals developed to atrium (ga) stage B are all large; and as stage B only represents a change in size of the atrium the effect is more likely to be due to growth than to a response to any special "sex organ stiumlation". The late stage marked in May for the separate parts of the tract, except the genital atrium, is due to one slug.

This one animal was collected casually (not at a trap on a collection night) on May 15 at the normal collecting place and was brought in because of its large size (5.6 gm) as compared to the field collections at the time. Unfortunately its genital atrium was ruined in processing. However, because of the late stages of the remainder of its tract it seems reasonable to suppose that this animal hatched the previous spring, had passed through the entire reproductive cycle, laying in early winter and overwintering.

June and July: the male glands are rapidly maturing at this time with a few of the larger animals practically ready for copulation by the end of the period.

August: most slugs are now in the mature male stage and a few are copulating. Only the very smallest animals (less than 3 gm) are now in earlier

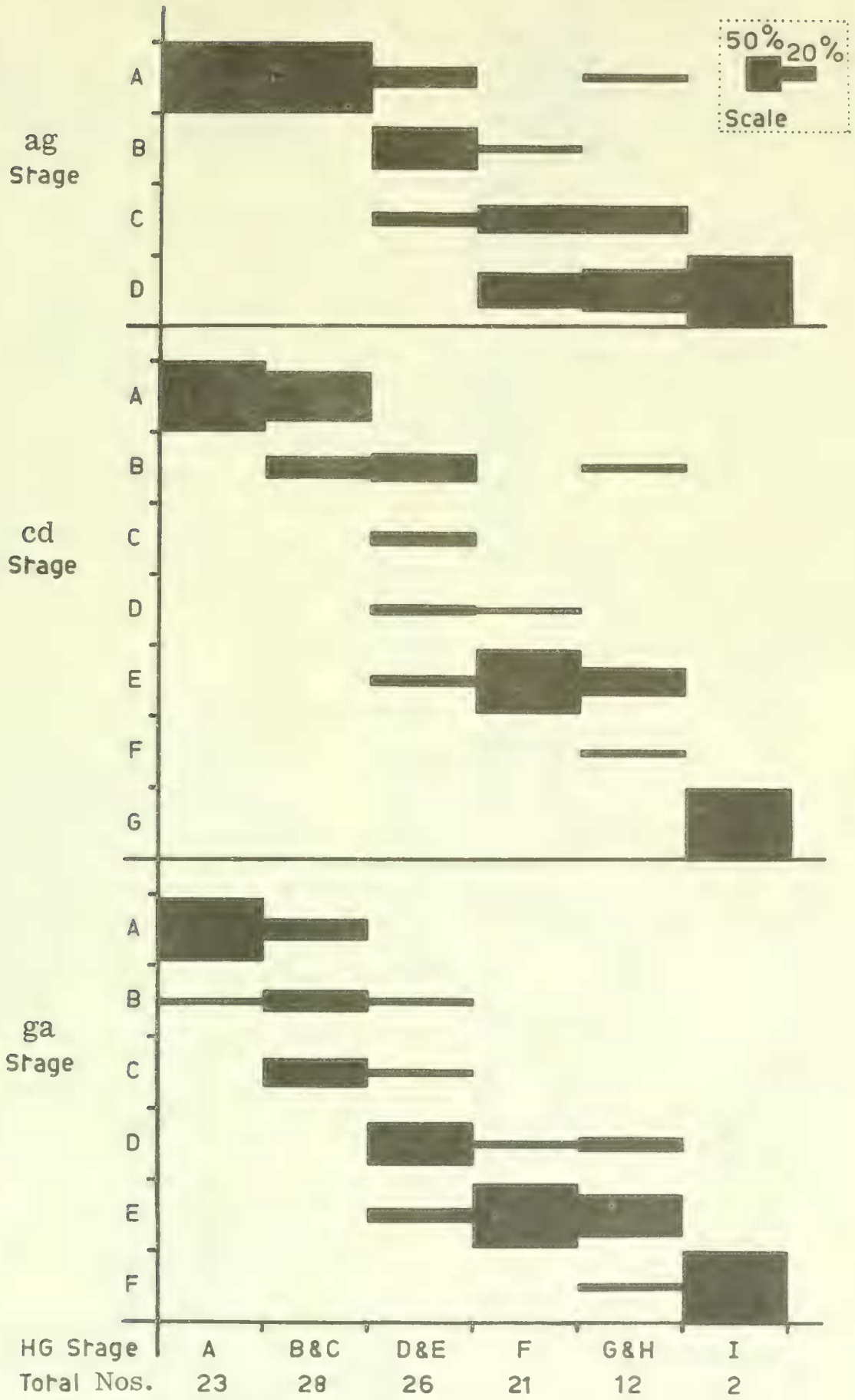

FIG. 8. Histogram showing the percentage of animals with each maturation stage of the albumen gland (ag), common duct (cd), and genital atria (ga) for each maturation stage of the hermaphrodite gland (hg).

stages. In a number of slugs the maturation of the female glands has begun.

September: copulation occurs widely and most of the animals have rapidly maturing female glands. One or two very small individuals are still in earlier stages.

October: nearly all the animals have copulated and a few of the large ones have started laying. The female glands in all the animals are now mature.

November: most animals are now laying or are in the post-laying atrophy stage. A few very small slugs with the reproductive organs in the very early stage of maturation are also present. These are newly hatched slugs from eggs laid that season.

COMPARISON OF THE HERMAPHRODITE GLAND STAGE WITH MATURATION OF THE REMAINDER OF THE REPRODUCTIVE TRACT

Laviolette (1954) has postulated that in *Arion subfuscus* the hermaphrodite gland controls the maturation of the remainder of the reproductive tract. An attempt has therefore been made to relate the stages of the various parts of the reproductive tract to the hermaphrodite gland stages, the results being illustrated in Fig. 8.

This histogram shows that most of the major changes in the maturation take place in the early and mid-spermatozoa stages (D and E) of the hermaphrodite gland. During these stages the female glands and the genital atria accessory glands all start rapidly to mature. Copulation occurs in the subsequent late spermatozoa stage (F) followed by egg laying and atrophy. The very early albumen gland (ag), common duct (cd) and atrial (ga) stages recorded for the oocyte stage (H and G) of the hermaphrodite gland represent one very small animal of 2.03 gm body weight. It is thus supposed that, although it was able to reach the oocyte stage in the hermaphrodite gland, it was too small to allow the rest of the tract to mature.

COMPARISON OF THE RATIOS OF REPRODUCTIVE TRACT REGION WEIGHT/BODY WEIGHT THROUGHOUT MATURATION

The weights of the different parts of the reproductive tract seemed to indicate a relationship between the body weight and the weights of the different regions of the tract at different stages of maturation. To test this impression the means of the percentages of the ratios of reproductive tract weight/body weight were compared by means of the "Student's t" test and the results tabulated in Table 1. It is seen that the sizes of the different regions of the reproductive tract compared to the body size--the differential growth rates--are, in the main, significantly different throughout the maturation cycle.

The hermaphrodite gland grows to a maximum size at the mid-spermatozoa stage and then is greatly reduced in size, due to the expulsion of the gametes. In the early stages the albumen gland and common duct were impossible to separate and, as the albumen gland was very small, the combined weight approximated well to the common duct weight. The large increase in weight of the tract at the onset of the female stage was largely due to the sudden enlargement of the albumen gland at that stage.

The results therefore tend to suggest that the groupings made in the description of the maturation cycle are real ones.

INVESTIGATION OF THE RELATIONSHIP OF MATURATION TO BODY WEIGHT

Lusis (1961) has suggested, for *Arion ater rufus*, that there is a direct relationship between maturation and body weight. The results of the present investigation do not support this idea.

To investigate this point the percentage of each hermaphrodite gland stage pre-

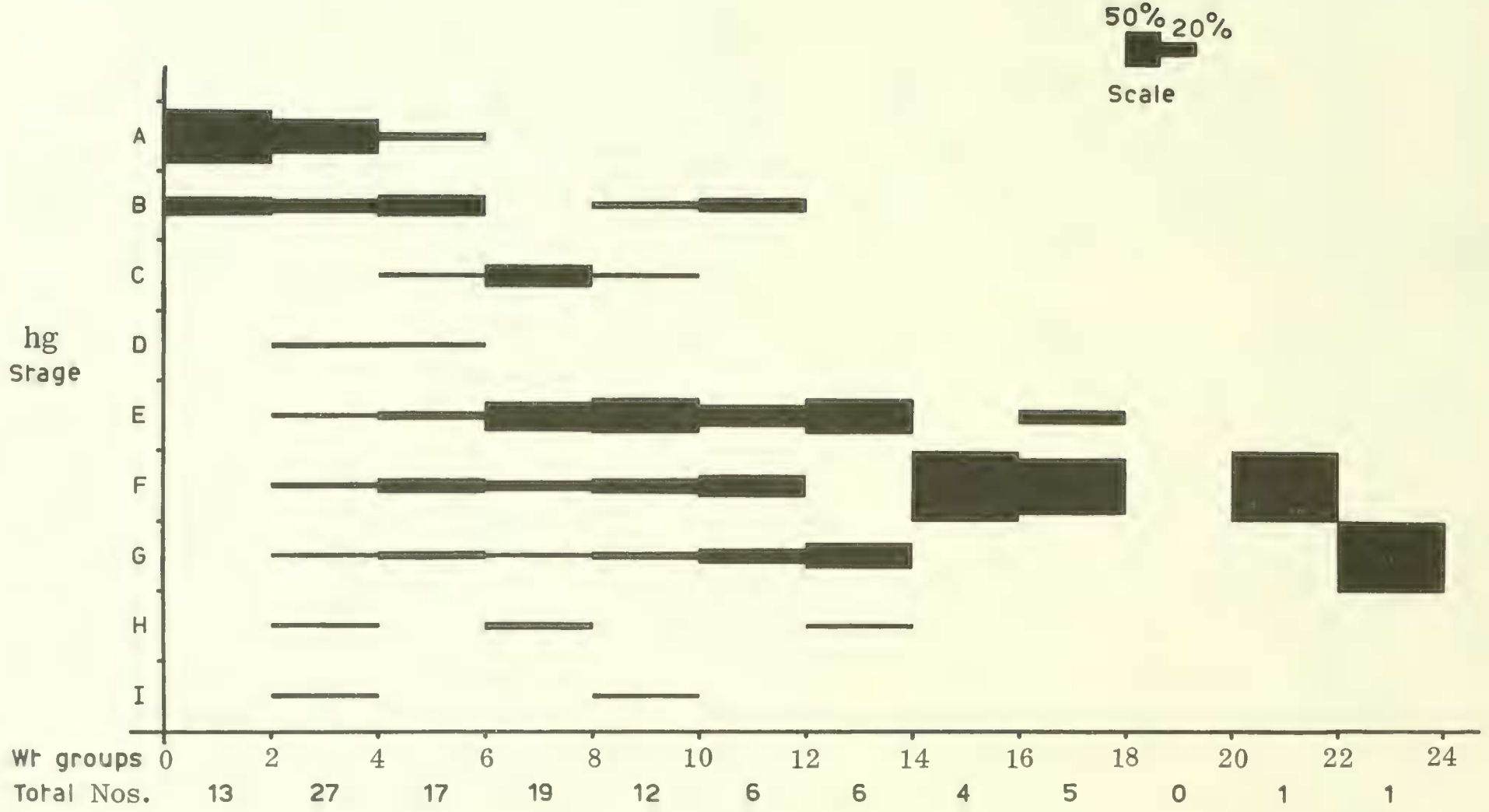

FIG. 9. Histogram showing the percentage of animals with each hermaphrodite gland maturation stage in each 2 gm body weight group.

TABLE 1. Comparison of the means of the percentages of the reproductive tract region weight/body weight at different stages of maturation by means of the "Student's t" test

Region of reproductive organs	Stages of development of the region*	No. of animals used	Mean of % Region wt. / body wt.	Value of "t"	Significance to 95% probability level
Hermaphrodite Gland	A	24	0.159		
	B and C	27	0.765	- 4.439	+
	D and E	22	2.930	- 9.448	+
	F	22	1.176	- 6.953	+
	C and H	14	0.618	- 2.793	+
Albumen Gland	B and C	32	3.996		
	D	20	9.430	- 4.990	+
Common Duct & Albumen Gland Common Duct	B of common duct	20	0.599		
	C & D of common duct	15	3.202	- 5.770	+
	E	34	4.867	- 5.761	+
Genital Atrium	B and C	27	0.611		
	D	19	2.496	- 8.409	+
	E and F	38	2.966	- 2.204	+
Whole of reproductive tract minus Hermaphrodite Gland	stages of herm. gland:				
	A	24	0.205	- 4.334	+
	B and C	27	0.387	-10.205	+
	D and E	22	4.820	-11.260	+
	F	22	15.602	- 0.504	-
	G and H	14	14.972		

*For stages see text.

sent in each 2 gm weight group was calculated (Fig. 9). The results show that while only the earlier stages are seen in the 0-2 gm weight group, all the other groups can contain all the maturation stages. The presence of only spermatozoa (E, F) and early oocyte (G) stages in the larger weight groups must be treated with extreme caution as these results are from very few (1-4)

TABLE 2. Comparison of mean body weights of 109 *Arion ater* of adjacent hermaphrodite gland stages of the "Student's t" test

Stage of hermaphrodite gland	No. of slugs used	Range of body weights g	Means of body weights g	Value of "t"	Significance to 95% probability level
A	24	0.79 - 4.76	2.525		
B and C	27	1.51 - 10.40	4.794	- 4.108	+
D and E	22	2.47 - 22.03	8.844	- 4.149	+
F	22	2.43 - 20.15	11.303	- 1.829	-
G and H	14	2.03 - 13.71	8.285	- 1.808	-

TABLE 3. Representative data for slugs hatched and reared in the laboratory at temperatures of 18–20°C with natural illumination

Date hatched	Date killed	Age in days	Body weight g	% Tract wt. / Body wt.	hg stage*
Nov. 2	Feb. 4	95	3.65	0.91	D
Nov. 2	Mar. 12	131	6.64	10.50	E
Nov. 26	Apr. 1	126	10.60	6.25	E
Nov. 2	Apr. 1	150	5.92	0.45	A
Nov. 26	Apr. 16	142	8.12	0.65	D
Nov. 26	May 28	181	12.23	9.63	E
Nov. 26	June 12	196	14.05	18.20	E
Nov. 26	June 12	196	4.97	0.14	A
Nov. 26	June 25	209	16.79	25.97	F
Nov. 26	June 25	209	11.48	0.59	D
Nov. 26	June 25	209	15.58	4.14	E
Nov. 2	Aug. 24	238	15.57	6.64	E
Nov. 2	Oct. 23	356	17.31	24.79	H
Nov. 26	Oct. 23	356	6.62	21.21	H

*See pp 329, 331 for key to hermaphrodite gland stage.

animals.

The means of the body weights of various hermaphrodite gland stages were compared by the "Student's t" test (Table 2). Even though the ranges of the body weights within each group are very big, this test may have some value. However, it is only in the earlier stages that some relationship might exist between body weight and maturation stage and even here the range of individual variation is so great as to almost overshadow any such relationship.

EXPERIMENTAL INVESTIGATION INTO FACTORS AFFECTING MATURATION

The experiments and their results are described below. The tables relating to the various experimental series do not comprise all slugs observed, but only a representative selection, each result quoted being representative of a group of similar animals.

Animals Hatched and Reared in the Laboratory

The slugs used in this experiment (about 60) were from 2 clutches of eggs hatched on November 2 and 26 and left in separate bowls. They were kept under fairly constant temperature conditions of 18-20°C and natural illumination. Data for a representative sample of these are given in Table 3. It is seen that, compared to slugs collected in the field (see Fig. 7) during the first half of the year, a number of experimental animals (about half of the 30 killed in that same period) showed an advancement of maturation, which seems to be correlated in some way with body size. These results agree with those of Lusis (1961) who also used animals hatched and reared in the laboratory. In addition, my slugs showed a great variation in body size, even though they were from the same clutch and kept in the same bowls. The relationship between body size and maturation disappeared as the natural breeding season approached.

Animals maintained at 18°C in natural illumination and darkness

For this experiment 25 slugs were kept in natural illumination and 15 in the dark. The representative data tabulated in Table 4 show that there seems to be a slight increase in body weight as com-

TABLE 4. Representative data for slugs kept at 18°C with (a) natural light (b) no light

Date collected	Date killed	Duration of experiment in days	Body weight g	% Tract wt. / Body wt.	hg stage*
(a)					
Mar. 17	May 31	57	11.18	0.85	C
Mar. 17	June 12	87	16.62	1.74	E
Mar. 17	June 25	100	12.80	3.42	E
Mar. 17	July 25	131	24.77	10.38	E
Mar. 17	Aug. 6	143	15.22	12.44	E
June 17	Aug. 26	70	18.59	5.42	E
July 17	Sept. 20	95	20.57	24.49	F
July 17	Sept. 20	95	12.63	14.38	F
(b)					
June 8	July 25	47	15.62	4.74	E
June 8	Aug. 22	75	21.54	4.83	E
June 8	Aug. 22	75	20.97	15.16	E

*See pp 329, 331 for key to hermaphrodite gland stage.

TABLE 5. Representative data for slugs kept at 10°C with (a) 16 hours of artificial light per day (b) no light

Date collected	Date killed	Duration of experiment in days	Body weight g	% Tract wt. / Body wt.	hg stage*
(a)					
Apr. 29	July 26	88	13.01	4.46	E
Apr. 29	Aug. 26	111	8.99	15.68	F
Apr. 29	Aug. 26	111	9.53	6.84	E
Apr. 29	Aug. 26	111	25.48	7.51	E
June 14	Aug. 27	74	10.52	23.33	F
June 14	Aug. 27	74	12.96	7.60	E
Apr. 29	Oct. 24	178	14.64	24.27	F
June 14	Oct. 24	132	18.85	36.96	H
(b)					
Apr. 29	July 26	88	13.52	0.89	E
Apr. 29	Aug. 27	120	13.74	4.63	E
Apr. 20	Aug. 27	129	7.17	1.55	D
June 14	Aug. 27	74	21.89	11.57	F
June 14	Aug. 27	74	11.16	3.79	E
Apr. 29	Oct. 24	178	15.43	40.10	H
Apr. 29	Oct. 24	178	8.94	3.30	E
June 14	Oct. 28	196	11.18	39.98	G
June 14	Oct. 28	196	11.78	5.45	E
June 14	Dec. 6	176	7.87	5.99	E
June 14	Dec. 6	176	20.04	32.88	H
June 14	Dec. 6	176	9.66	1.46	D

*See pp 329, 331 for key to hermaphrodite gland stage.

TABLE 6. Representative data for slugs kept at 4°C with 16 hours of artificial light per day

Date collected	Date killed	Duration of experiment in days	Body weight g	% Tract wt. / Body wt.	hg stage*
Apr. 29	July 26	88	6.88	0.20	C
Apr. 29	Aug. 20	113	5.98	0.59	D
Apr. 29	Aug. 20	113	2.47	0.24	A
Apr. 29	Aug. 20	113	4.74	0.61	D
Apr. 29	Aug. 20	113	3.73	0.24	B
June 14	Aug. 29	75	8.30	0.53	D
June 14	Aug. 28	75	6.89	0.49	C
June 14	Oct. 24	132	8.51	3.36	E
June 14	Oct. 24	132	9.28	3.71	E
July 9	Oct. 28	111	5.54	4.26	D
Sept. 29	Oct. 28	29	11.85	29.63	G
Sept. 29	Oct. 28	29	9.16	22.03	G
June 14	Oct. 28	136	5.49	2.53	E
June 14	Oct. 28	136	5.05	0.79	D
June 14	Oct. 28	136	2.91	0.27	C

*See pp 329., 331 for key to hermaphrodite gland stage.

pared to animals from the natural population over the same period (see Fig. 1). The slugs also seem to start maturing a little earlier, although the period of maturation does not appear to be shorter. The same also holds true for all the animals left in the dark. These results seem to derive from the nearly optimal conditions.

A relationship between food and general conditions and maturation is shown by 4 animals (not included in the table) which were collected on August 12 as extremely small immature specimens, kept in the laboratory without food in a bowl with increasingly decaying vegatation and killed on December 6. They showed no body or reproductive growth.

Animals maintained at 10°C with 16 hours of light per day and in darkness

Two groups of 50 slugs, with half of each group from 2 different collections, were kept in each of the illumination conditions. Details for a representative sample are tabulated in Table 5. At this low temperature the maturation cycle slowed down slightly, especially in the commencement of the female stage (F). This retardation was particularly evident in animals maintained in the dark, but is probably a temperature response. When the slugs were exposed to artificial light for 16 hrs/day the temperature fluctuated between 14° and 9°C, due to the heating effect of the lights, whereas, when they were maintained without light, the temperature remained more constantly equal to the minimum reading. Exceptions to the general slowing down of maturation were observed in large animals.

The 3 slugs killed on December 6 showed that while prolonged exposure to low temperature retarded the 2 small individuals in their maturation, the large animal was unaffected. This latter aspect will be discussed later. In one of the smaller slugs the long exposure had produced abnormalities in the hermaphrodite gland. The alveoli were very small, with thick walls and large cells having long finger-like processes projecting into the lumina—all characteristics of a late oocyte or atrophy stage. The centres of the lumina were, however,

TABLE 7. Representative data for slugs kept at 25°C with (a) 16 hours of artificial light per day (b) no light

Date collected	Date killed	Duration of experiment in days	Body weight g	% Tract wt. / Body wt.	hg stage*
(a)					
May 7	May 31	24	3.35	0.21	B
June 23	July 27	34	8.38	0.41	C
June 23	July 29	36	6.66	0.28	C
(b)					
Apr. 29	May 31	33	2.63	0.15	B
June 9	July 27	48	7.96	0.56	C
June 9	July 29	50	6.69	0.33	B
June 9	July 29	50	7.82	0.41	B
Sept. 29	Oct. 23	24	6.66	24.76	G
Sept. 29	Oct. 23	24	5.61	2.58	C
Sept. 29	Oct. 23	24	4.67	2.40	D

*See pp 329, 331 for key to hermaphrodite gland stage.

full of spermatids and a few developing spermatozoa, and it must therefore be classified as stage D.

Animals maintained at 4°C and 16 hours of light per day

This experiment was carried out with 45 slugs. Details for a representative sample are given in Table 6. The effect of exposure to the extremely low temperature was to almost arrest development at the stage in which the animal was at the beginning of the experimental period. Once or twice during the experiments the temperature dropped well below freezing for a short time and although most of the animals recovered (less than 5% mortality) the excessive cold may have adversely affected them. About half of the slugs had hermaphrodite glands with abnormalities, i.e., they had many characteristics of oocyte stages, while actually being in the early or mid-spermatozoa stages.

Animals maintained at 25°C in 16 hours of light and in darkness

The experiment was carried out with 8 groups of 15 slugs starting at various times. At this high temperature there was a very high mortality especially in the first month, before accurate temperature control had been fitted. Whenever the temperature rose to 28°C or above, all the animals died quickly. The mortality throughout all the experiments in this group was about 60%. Detailed results for a representative sample are tabulated in Table 7. The effect of the high temperature on the animals that did survive was to almost completely stop the maturation of the reproductive organs. Most animals, especially those left in the dark, showed gross abnormalities in the hermaphrodite gland: in a large number of alveoli resorption of all cells, usually associated with the oocyte stage, was observed. However, the presence of less abnormal areas made accurate classification possible.

Animals maintained in continuous light at 20°C

Although the temperature was maintained fairly constantly, there was a moderate mortality of about 50% among the 25 slugs used in this experiment. The effect on survivors was to slow down

TABLE 8. Representative data for slugs kept at 20°C, with continuous artificial light

Date collected	Date killed	Duration of experiment in days	Body weight g	% Trace wt. / Body wt.	hg stage*
June 14	July 27	43	10.74	0.40	C
June 14	July 27	43	11.24	0.91	D
June 14	Aug. 17	64	10.25	1.67	E
June 14	Aug. 17	64	14.47	3.70	D
June 14	Aug. 17	64	14.17	1.70	D
June 14	Aug. 17	64	10.68	3.17	E

*See pp 329, 331 for key to hermaphrodite gland stage.

the maturation of the reproductive tract, as exemplified in the detailed data on a representative sample tabulated in Table 8. These conditions also caused a partial resorption of the cells in the alveoli expecially of mature sperm.

Animals maintained with 8 hours of light per day at 18°C

The experiment was carried out with 25 slugs. The restricted day length had no noticeable effect on the maturation of the reproductive organs and the only noticeable difference from the field slugs was a very slight increase in average body size probably due to the more favourable conditions.

DISCUSSION AND CONCLUSIONS

The function and histochemistry of the various glands of the reproductive tract of *Arion ater* have been described by the author (Smith, 1964, 1965). It was concluded that the secretions of the male flask gland and prostate gland combined to form the spermatophore material, that the albumen gland secreted the perivitelline fluid, that the two-layered egg shell was secreted by the female gland and amoebocytes in the free oviduct.

Lusis (1961) suggested that the activity of the reproductive tract of *Arion ater* is related to the age of the animal and to body size. From his results, however, it is seen that animals enter the oocyte stage of maturation at ages ranging from 6-12 months. Moreover, he got such a wide body-weight range, even within one maturation stage, that these correlations must be treated with caution.

Laviolette (1954) has postulated that the hermaphrodite gland controls the maturation of the reproductive tract of *Arion subfuscus* by means of a hormone, though the exact site of its production in the gland is not known. This present investigation is in agreement with his view in so far as each hermaphrodite gland stage has a corresponding tract gland stage. The "spermatid stage" is connected with the commencement of maturation of the male glands. The "early spermatozoa stage" sees the maturity of the male glands and the differentiation and commencement of maturation of the female glands and all the accessory structures associated with copulation. The "late spermatozoa stage" is connected with copulation and the "oocyte stage" with the maturation of the female gland and with egg laying. These relationships between the hermaphrodite gland and the remainder of the tract always exist in the animals weighing more than about 2 gm. One 2 gm animal, however, possessed a hermaphrodite gland in the early oocyte stage while the remainder of its reproductive tract was very immature. It might be possible that for some reason, the tract of an animal of such small size cannot attain maturity. Presumably animals collected from the field are of

approximately the same age and it could be possible that the maturation stages of both the hermaphrodite gland and the tract are either independently related to age or are inter-dependent. That the latter alternative holds true is, however, proved by those experimental animals, in which the rate of maturation of the reproductive tract had been changed. In all these cases there exists this same relationship between hermaphrodite gland stage and the stage of maturation of the remainder of the tract. There would seem to be 2 possible explanations of this constant relationship. Firstly, both the hermaphrodite gland and the rest of the reproductive tract could be under one common control mechanism. Alternatively it is possible that one region controls the other. From Laviolette's (1954) experiments on transplantation of reproductive tracts from one maturation stage to another in *Arion* sp., it would appear that the hermaphrodite gland does control the maturation of the rest of the tract. I was however unable to confirm these results.

The connection between the maturation of the hermaphrodite gland and the season, shown in Fig. 7, indicates a response to some controlling factor or factors in the environment. This relationship is, however, not absolute, as it is modified slightly according to body size, the larger animals being more advanced. This tends to suggest that the effect of external factors is not direct, but must act through other controlling links.

In experiments to investigate the effect of different environmental factors on animals collected from a wild population, the advancement of maturation of the reproductive tract under favourable conditions such as temperatures of 18°C, natural illumination or total darkness and plenty of fresh food daily, was only marginal, about a month or 6 weeks. This advance may have been almost entirely due to the larger size of the animals brought about by optimum conditions. Using animals hatched and reared in the laboratory brought about an advancement of up to 7 months, animals being already in the female stage when the whole of the wild population was still in the spermatocyte stage. This extremely premature maturation cannot be associated with body size for, although these animals were amongst the largest in the bowls at the time, they were still small when compared with the average size of animals in the female stage at the normal period. It must also be noted that while some animals showed advancement, others from the same clutch showed little or no sign of it. The mechanism of this unusual advancement of maturation is unknown, although it may be due to constant conditions or the lack of an over-wintering rest period for the eggs or young.

In experiments where the maturation was retarded by extremely low or high temperatures particularly, animals usually showed some degree of abnormality in the hermaphrodite gland, which was usually caused by resorption of some or all of the products of gametogenesis and was, in every case, associated with a small immature reproductive tract. It thus seems that the experimental conditions affect the metabolism of the animals and so affect maturation. In most of these groups however some animals were found, usually large ones, which seem virtually unaffected by the conditions. These animals were all seen late in the year and were in the normal stage for that period, i.e., in the female stage.

From these results the maturation of the reproductive tract of *Arion ater* is seen to be divisible into 3 phases:

1) Differentiation of the male glands: this phase begins in early spring and is connected with the onset of spermatogenesis; it is probably initiated in some way by the general rise in temperature at that time. After the onset of this phase the maturation of the male part of the tract then proceeds in relation to the general metabolic rate.

2) Copulation and differentiation of the female glands: the onset of this phase occurs during the mid-spermato-

zoa stage (E) of the hermaphrodite gland in July or August. It marks a "critical point" in the maturation of the reproductive organs, at which a large number of major changes take place. The genital atria and their several accessory glands rapidly mature and the female glands begin to differentiate. This is the time when mature spermatozoa are beginning to be shed into the hermaphrodite duct and this release may be the initial trigger for the entire phase, since it has been shown that the hermaphrodite gland in some way holds some controlling influence over the remainder of the tract. From observations on some of the experimental animals it is seen that, once this "critical point" has been passed, adverse external conditions have very little effect on the subsequent maturation of the reproductive organs, while they have a profound effect before this point. These results suggest a "trigger" mechanism of control which is probably of a neurosecretory nature, as described by the author (Smith, 1964). From the "critical point" onwards the rate of maturation of the female glands and also the time of copulation depends upon the general metabolic rate.

3) Fertilization, laying and the onset of atrophy: these 3 processes are closely interconnected, as fertilization is the first phase of the laying process (Smith, 1964, 1965) and atrophy begins with the emptying of the gland cells during shell formation during laying. These processes probably occur at a standard period after copulation, again depending on general metabolic rate.

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RESUMEN

MADURACION DEL CANAL REPRODUCTOR DE *ARION ATER* (PULMONATA)

La maduración del tracto reproductor de la babosa de huertas, *Arion ater* L., y los posibles factores influyentes en ella, fueron estudiados. Las babosas se recogieron de una población natural a intervalos regulares en toda la época de crianza y divididas en grupos por peso: los canales reproductores fueron disectados y las varias partes pesadas y clasificadas histológicamente por estados de madurez.

Observaciones en el campo mostraron que muy pocos animales consiguen superar el invierno despues de dar cria, pero que son capaces de hacerlo cuando en el huevo

o en estados muy jovenes. Crecimiento tiene lugar durante todo el año y hay siempre amplia variación de tamaño en una determinada población. En Inglaterra la copulación ocurre de julio a septiembre y muy pronto es seguido por las puesta. Una gran disminución ocurre en el campo, probablemente debido a mortalidad post-desovadora.

Las varias partes del aparato reproductor pueden ser facilmente dividias en un número de estados de maduración reconocibles: glándula hermafrodita 9 estados; glándula albuminoidea 4 estados; ducto común 7 estados; atrio genital, epifalo y espermateca 6 estados; todos estos son descriptos en detalle. Existe una general relación entre la madurez del canal y la estación, y la maduración de la glándula hermafrodita esta correlacionada a la del resto del canal. Las proporciones (ratios) del peso de la región reproductora/peso del cuerpo, para cada estado de madurez, resultó significativamente diferente, mostrando que la división por grupos era natural. Final y contrariamente a la sugestión de Lusis (1961), muy poca relación existe entre madurez y tamaño del cuerpo.

Animales colectados de la misma población natural, se sometieron a factores de ambiente diferentes y controlados, para comparación con los primeros. Estos eran, temperaturas casi constantes de 4^{o}, 10^{o}, 18^{o} y 25^{o}C por periodos variables y tambien luz artifical por 8 y 16 horas diarias y continuamente, a luz de día completo y a obscuridad. Aunque el ciclo reproductivo puede adelantarse o retardarse por estos factores, la relación entre el estado de madurez de la glándula hermafrodita y el resto del canal no se altera. Animales eclosionados y criados en el laboratorio mostraron una grosera variación en su maduración, posiblemente debido a alguna irregularidad en sus mecanismos de control.

Se pueden distinguir tres fases de maduración en el aparato reproductor de *Arion ater:*

1. Diferenciación de las glándulas masculinas, que se inicia en primavera con al ascenso de temperatura.
2. Cópula, y diferenciación de las glándulas femeninas, la cual ocurre durante el estado espermatozoico-medio de la glándula hermafrodita entre julio y septiembre y es el "punto crítico" de maduración. Un mecanismo "disparador" de control, probablemente de naturaleza neurosecretora, se postula como ya lo sugiriera el autor (Smith, 1964).
3. Fertilización, puesta y comienzo de atrofia, posiblemente a un periodo standard despues de la copulación y dependiente del indice metabólico.

АБСТРАКТ

РЕПРОДУКТИВНЫЙ ТРАКТ СЛИЗНЯ *ARION ATER* (PULMONATA: ARIONIDAE)

Б. И. Смит

Процесс созревания репродуктивного тракта у садового слизня *Arion ater L.* был детально исследован как и факты, влияющие на него.

Слизни были собраны из естественных популяций через регулярные промежутки времени, во время сезона их размножения, и разделены были на весовые группы; их репродуктивные тракты были вскрыты, различные части были взвешены и гистологически разделены на стадии созревания.

Наблюдения в поле показали, что очень немногие животные могут пережить зиму после совокупления, но они легко перезимуют в стадиях яйца или в стадиях постэмбриональных. Рост их продолжается весь год и в каждой популяции всегда находятся все стадии роста. В Англии копуляция их происходит от июля до сентября и вскоре следует откладка яиц. После этого наблюдается резкое понижение в количествах каждой популяции, ве-

роятно вследствие смертности после откладки.

Различные части репродуктивного тракта могут быть разделены на несколько легко отличимых стадий полового вызревания: гермафродитная железа; 9 стадий; эндоспермная железа: 4 стадии; семепровод: 7 стадий, половые атрий, эпифаллус и сперматека: 6 стадий, которые детально описаны. Было установлено, что созревание тракта и сезон созревания гермафродитной железы находятся в тесной зависимости от вызревания остальной части тракта. Взвешивания репродуктивного тракта и всего тела для каждой стадии дало различные пропорции, как бы подтверждая их действительность. Но зависимость между половым созреванием и размерами тела была незначительной, в противоположность предположению, высказанному Луисом(1961). Собранные в тех же естественных популяциях животные были подвержены различным контрольным экологическим факторам и сравнивались с особями из естественных популяций. Они были подвержены почти постоянной температуре в 4, 10, 18 и 28 градусов на различные периоды времени, а также искусственному свету на 8 и на 16 часов в сутки и постоянному свету, т.е. естественному дневному свету и вовсе без света. Хотя репродуктивные циклы могли быть задержаны или ускорены в таких условиях, но зависимость созревания гермафродитной железы и всего репродуктивного тракта не могла быть нарушена. Вылупившиеся и выращенные в лаборатории животные дали большое разнообразие в процессе созревания, вероятно, из за расстройства их контрольного механизма.

В созревании репродуктивного тракта *A. ater* можно различить три фазы:

1. Видоизменение мужских желез, стимулированное общим повышением температуры весной.

2. Копуляция и видоизменение женских желез, происходящие в половине сперматозоидной стадии гермафродитной железы между июлем и сентябрем, что является "критическим моментом" созревания. Контрольный механизм, напоминающий "защелку", вероятно, нервной секреции обуславливает эту стадию, как это ранее предполагал Смит (1964).

3. Оплодотворение, яйцекладка и атрофия, возможно, следуют через определенное время после копуляции и в зависимости от состояния общего метаболизма.